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Succinic acid fermentation in a stationary-basket bioreactor with a packed bed of immobilized *Actinobacillus succinogenes*: 1. Influence of internal diffusion on substrate mass transfer and consumption rate

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Abstract This paper is dedicated to the study on external and internal mass transfers of glucose for succinic fermentation under substrate and product inhibitions using a bioreactor with a stationary basket bed of immobilized *Actinobacillus succinogenes* cells. By means of the substrate mass balance for a single particle of biocatalysts, considering the Jerusalimsky kinetic model including both inhibitory effects, specific mathematical expressions have been developed for describing the profiles of the substrate concentrations and mass flows in the outer and inner regions of biocatalyst particles, as well as for estimating the influence of internal diffusion on glucose consumption rate. The results indicated that very low values of internal mass flow could be reached in the particles center. The corresponding region was considered biologically inactive, with its extent varying from 0.24% to 44% from the overall volume of each biocatalyst. By immobilization of bacterial cells and use of a basket bed, the rate of glucose consumption is reduced up to 200 times compared with the succinic fermentation system containing free cells.

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Introduction

Succinic acid has numerous applications in the chemical industry (reagents, synthetic resins, biodegradable polymers, electroplating, green solvents, inks), agriculture (pesticides, growth regulators and stimulators), pharmaceutical and food industries (amino acids, antibiotics, vitamins, surfactants, additives) [17, 23, 29, 33]. This dicarboxylic acid is industrially produced using liquefied petroleum gas—namely, butane—by chemical synthesis via maleic anhydride [33]. The cost of this technology could reach 6.3 euro/kg succinic acid depending on the acid's final purity, the contribution of raw material to this cost varying between 16% and 24% [17, 33]. Moreover, the downstream processes and chemical technology create important problems concerning environmental protection [17].

For the above reasons, a large number of microorganisms, which are potentially producers of succinic acid, has been tested: bacteria (*Veillonella parvula, Selenomonas ruminantium*, *Succiniclasticus ruminis*, *Corynebacterium glutamicum, Enterococcus fecalis*, *Actinobacillus succinogenes*, *A. succiniproducens*, *Mannheimia succiniproducens*, *Escherichia coli*) [4, 6, 13, 15, 30, 31], yeasts (*Saccharomyces cerevisiae*) [1], and fungus (*Aspergillus niger*, *A. fumigatus*, *Byssochlamys nivea*, *Lentinus degener*, *Paecilomyces varioti*, *Penicillium viniferum*) [5, 16]. However, due either to the low yield of substrate bioconversion into succinic acid or to the non-Newtonian rheology and complex composition of the final broth, only *A.*

succinogenes and *A. succiniproducens* have been considered as important producers of this acid. These microorganisms possess the ability to convert various carbon sources (glucose, saccharose, molasses, glycerol, starch, cellulosic hydrolysates, or milling byproducts) under anaerobic conditions into succinic acid and secondary acids (formic, acetic, pyruvic acids) [6, 15, 17, 26, 31].

Most fermentative systems for succinic acid production have been carried out using free *A. succinogenes* cells, the process being affected by the substrate and product inhibition phenomena [8, 10]. Except for our previous works [10], there is no information concerning the use of immobilized *A. succinogenes*. The use of immobilized microorganisms or enzymes offers the advantages of the increase of thermal, chemical, and shear-force resistance of the biocatalysts. Other advantages consist of diminution or avoidance of inhibition processes, easier recovery of biocatalysts from final broths, and, consequently, increase in the number of repeated biosynthesis cycles reusing the same particles of biocatalysts [8]. Bioreactors using immobilized biocatalysts can be designed as column, stirred, gas-lift, or membrane bioreactors, being operated in batch, continuous, or semicontinuous systems, with fixed, mobile/stirred, expanded, or fluidized beds [10]. Although the bioreactors with a fixed bed of biocatalysts are widely preferred, they have some major disadvantages [8]. The flow inside the bed is laminar, thus leading to low rates of mass and heat transfer and inducing the back-mixing or reverse-flow phenomenon. On the other hand, the solid particles from effluent can clog the biocatalyst bed, thus leading to reduced flow rate inside the bed and biocatalysts inactivation. Another important undesirable phenomenon is formation of preferential flow channels inside the bed at the beginning of the medium feed or while the bioreactor is working. The formation of these channels induces deviation from the plug flow and inefficient conversion of the substrate.

Basket-type bioreactors are derived from bioreactors with fixed beds, the biocatalyst particles being fixed in an annular cylindrical or conic bed, which is either static around the stirrer [11, 14, 20] or rotary [22, 24, 27]. Owing to its design, this bioreactor avoids the disadvantages of bioreactors with fixed beds and flooding/deposition or mechanical disruption of biocatalysts particles, phenomena which are encountered in the bioreactors with mobile beds. In this bioreactor, the liquid phase flow combines the perfect mixed flow around the basket, with plug flow inside the biocatalysts bed. Thus, hydrodynamics of the medium around the basket exhibit an important influence on the transfer processes involved in the substrate conversion.

Previous studies indicate that the immobilization of yeast or bacterial cells in alginate and their use in systems with a stirred bed of biocatalysts can represent a viable alternative to the fermentation with free cells [8]. By selecting the optimum operating regime of the bioreactor, the activity and physical integrity of immobilized cells remain unaffected for many fermentation cycles, even if fermentation is carried out under substrate or product inhibition conditions [8]. In this context, our work investigates the external and internal mass transfer of glucose, under substrate and product inhibition limitations, in succinic acid production by *A. succinogenes* immobilized using a stationary basket bioreactor. Based on the experimental results, new mathematical models quantifying the implication of internal diffusion on the profile of substrate concentration in the outer region and inside the biocatalyst particle, as well as on the reduction of substrate consumption rate, have been established.

Materials and method

Mathematical model for substrate diffusion

The values of glucose concentrations at the particle surface and inside the particle can be obtained by means of its mass balance related to a single biocatalyst particle. To this purpose, the following assumptions have been considered:

- the kinetics of succinic acid production can be described by a Jerusalimsky model for substrate and product inhibition, adapted to the immobilized *A. succinogenes* cells [4]:

$$v_P = V \cdot C_C \cdot \left(\frac{K_{iS}}{K_{iS} + C_S}\right) \cdot \left(\frac{K_{iP}}{K_{iP} + Y_{P/S} \cdot C_S}\right) \quad (1)$$

- the biocatalyst particle is spherical;
- the yeast cells are uniformly distributed inside the particle;
- there are no interactions between the substrate or products and support;
- the internal diffusion is described by Fick law and effective diffusivity.

In this case, according to the Bird equation [2], expression for the mass balance of glucose related to the biocatalyst particle is:

$$\frac{dC_{SP}}{dt} = D_{Se} \cdot \left[\frac{1}{r^2} \cdot \frac{d}{dr}\left(r^2 \cdot \frac{dC_{SP}}{dr}\right)\right] - V \cdot C_C \cdot \left(\frac{K_{iS}}{K_{iS} + C_{SP}}\right) \cdot \left(\frac{K_{iP}}{K_{iP} + Y_{P/S} \cdot C_{SP}}\right) \quad (2)$$

where: C_C, cells concentration (kg/m^3 dw); C_S, substrate concentration (kg/m^3); C_{SP}, substrate concentration inside the biocatalyst particle (kg/m^3); D_{Se}, effective diffusivity (m^2/s); K_{iP}, product inhibition constant (kg/m^3); K_{iS}, substrate inhibition constant (kg/m^3); V, maximum

biochemical reaction rate (kg/kg s); $Y_{P/S}$, yield of substrate conversion to product (kg/kg).

Considering the steady-state conditions, Eq. 2 becomes:

$$\frac{d^2C_{SP}}{dr^2}+\frac{2}{r}\cdot\frac{dC_{SP}}{dr}=\frac{V\cdot C_C}{D_{Se}}\cdot\left(\frac{K_{iS}}{K_{iS}+C_{SP}}\right)\cdot\left(\frac{K_{iP}}{K_{iP}+Y_{P/S}\cdot C_{SP}}\right) \quad (3)$$

and can be solved under the following boundary limits:

1. $r = 0$ (at particle center), $\frac{dC_{SP}}{dr}=0$

2. $r = R_P$ (at particle surface), $-D_{Se}\cdot\frac{dC_{SP}}{dr}=k_L\cdot(C_{SL}-C_{Si})$

k_L being the liquid phase mass transfer coefficient (m/s).

The solution of Eq. 2 was determined using the Prelle-Singer method for finding the first integrals, the closed-form solutions of first-order differential equations, as well as Kovacic's results on second-order linear ordinary differential equations [21]. This model describes the dynamics of compartmentalized processes, the input of which is substrate concentration in the liquid phase, C_{SL}, and outputs substrate concentrations inside, C_{SP}, and at the surface of the biocatalyst particle, C_{Si}.

In these circumstances, the solutions of Eq. 3 describe the glucose concentration profile inside the biocatalyst particle, C_{SP}, with radius R_P:

$$C_{SP}=\frac{Bi\cdot(C_{SL}-C_{Si})\cdot\cosh(3\varphi\cdot R_p)}{R_P^2}\cdot\left[\frac{3\varphi}{R_p}-R_p\cdot\tanh(3\varphi\cdot R_p)\right]\cdot\frac{\sinh(3\varphi\cdot r)}{r} \quad (4)$$

and the substrate concentration at the particle surface, C_{Si}:

$$C_{Si}=\frac{Bi\cdot C_{SL}\cdot\cosh(3\varphi\cdot R_p)\cdot\left[3\varphi-R_p^2\cdot\tanh(3\varphi\cdot R_p)\right]\cdot\sinh(3\varphi)-C_{SL}\cdot R_p^4}{Bi\cdot\cosh(3\varphi\cdot R_p)\cdot\left[3\varphi-R_p^2\cdot\tanh(3\varphi\cdot R_p)\right]} \quad (5)$$

(C_{SL} represents the substrate concentration in liquid phase, kg/m^3).

The Thiele modulus, φ, and the Biot number, Bi, quantify the influence of the internal diffusion. Thus, the Thiele modulus indicates the magnitude of the influence of internal diffusion on the biochemical reaction rate [9]. For the studied fermentation system, the Thiele modulus is defined by the modified expression [10]:

Table 1 Parameters used for calculations

Parameter	Value	Source
D_{SL} (m^2/s)	6.47×10^{-10}	[25]
D_{Se} (m^2/s)	4.39×10^{-10}	[7]
K_{Is} (kg/m^3)	80	[4]
K_{iP} (kg/m^3)	48	[4]
V (kg/kg s)	1.75×10^{-4}	[4]
$Y_{P/S}$ (kg/kg)	1.10	[15]

$$\varphi=\frac{R_p}{3}\cdot\sqrt{\frac{V\cdot C_C}{D_{Se}}\cdot\frac{Y_{P/S}\cdot K_{iS}+1}{K_{iP}\cdot K_{iS}}} \quad (6)$$

The Biot number represents the ratio between resistance to the diffusion in the boundary layer surrounding the particle and that corresponding to the internal diffusion:

$$Bi=\frac{k_L\cdot R_P}{D_{Se}} \quad (7)$$

The values of the parameters used for calculations have been previously established and are given in Table 1.

Equipment, materials, microorganism

The experiments were carried out in batch system in 10 l (8 l working volume) laboratory-stirred bioreactor Fermac 310/60 (Electrolab) [3]. The bioreactor was provided with a cylindrical bed of the basket type, with the inner diameter 100 mm, height 100 mm, and bed thickness 10 mm. The basket was placed centered around the stirrer at 100 mm from the bioreactor bottom. According to previous studies, the optimum impeller combination was found to be of two Rushton turbines, the superior one placed outside the basket and the other inside the basket at its inferior extremity [18]. The impeller rotation speed was 150. The basket was filed with *A. succinogenes* ATCC 55617 cells immobilized in alginate. The microorganism was provided by the American Type Culture Collection and preserved at −70°C. The inoculum was prepared by incubating *A. succinogenes* at 30°C in 100 ml Duran bottles, each containing 50 ml trypticase soya broth. The bottles were stirred at 100 rpm on a

rotary shaker for 48 h. Immobilization was carried out by adding bacterial cells to the alginate matrix according to the method given in literature [28]. The biocatalysts were prepared in aseptic conditions. For this purpose, 6 ml of inoculum was mixed with 20 ml of 5% aqueous solution of sodium alginate. The biocatalysts particles were obtained by dripping this suspension through a capillary into a solution of 0.2% calcium chloride ($CaCl_2$) under constant pressure. Capillaries with three different diameters were used, and the obtained particles of immobilized *A. succinogenes* had the following diameters: 3.0, 3.6, and 4.2 mm, respectively. Any mechanical damage to the biocatalyst due to shear forces was recorded during the experiments.

Medium composition was glucose 30 g; yeast extract 5 g; $NaH_2PO_4{\cdot}H_2O$) 1.16 g; Na_2HPO_4 0.31 g; NaCl 1.0 g; $MgCl_2{\cdot}6H_2O$ 0.2 g; $CaCl_2{\cdot}2H_2O$, 0.2 g; vitamin B_{12}, 1 μg; biotin, 20 μg; folic acid, 20 μg; thiamine, 50 μg; riboflavin, 50 μg; niacin, 50 μg; pantothenate, 50 μg; *p*-aminobenzoate, 50 μg; lipoic acid, 50 μg; vitamin B_6, 100 μg; $MgCO_3$, 30 g; silicone antifoam, 1 ml [15]. The fermentation temperature was 37°C.

Experimental values of the external mass transfer rate were calculated and analyzed by means of the variation of glucose concentrations in the liquid bulk volume and biocatalysts particle surface during fermentation. The location of each sampling point was 150 and 100 mm from the bioreactor bottom, as follows: one in the inner region of the cylindrical bed, one at the inner and other at the outer surfaces of the cylindrical bed, and three inside the cylindrical bed (two sampling points each at 25 mm from the two surfaces of the cylinder and one in the middle of the bed thickness). Glucose concentration was measured using high-performance liquid chromatography (HPLC) with a Phenomenex Rezex ROA column (7.8 mm diameter, 300 mm length) provided with the refractive index detector RID-10A. The mobile phase was a solution of 5×10^{-3} N sulfuric acid with a flow rate of 0.6 ml/min. Analysis temperature was of 65°C. Internal values of glucose concentration or mass transfer were calculated using only the proposed mathematical model. The fermentation end was considered when either the glucose was completely consumed or its concentration remained constant for 12 h. Because the fermentation process is practically finished after 24 h [15], the samples were taken at 5, 10, 15 and 20 h from the process beginning. Each experiment was repeated two or three times under identical conditions, with the average value of the considered parameters being used. The maximum experimental error was of ±6.08%.

Results and discussion

Internal diffusion is important, especially for the biocatalysts immobilized inside an inert matrix. In this case, the substrate must migrate to the cells or enzymes through nonlinear channels, its diffusion being described by the effective diffusion coefficient or diffusivity. The rate of the biochemical reactions occurring inside the biocatalyst particle is inferior to that corresponding to the homogeneous system due to the lower substrate concentration compared with its value in the liquid bulk. Using substrate concentration values in the liquid bulk and inside the biocatalyst particle, the external and internal mass flows of glucose can be calculated. Therefore, the substrate flux from the liquid phase to the particle surface is:

$$n_L = k_L \cdot (C_{SL} - C_{Si}) \tag{8}$$

where k_L represents the mass transfer coefficient in the boundary layer at the particle surface, being calculated with the expression adequate for the fixed bed [19]:

$$\frac{\frac{k_L \cdot d_P}{D_{SL}} \cdot \frac{\eta_L}{v_s \cdot \rho_L \cdot d_P}}{\left(\frac{\eta_L}{\rho_L \cdot D_{SL}}\right)^{1/3}} = 5,7 \cdot \left[\frac{(1-\phi)\cdot \eta_L}{v_s \cdot \rho_L \cdot d_P}\right]^{0.78} \tag{9}$$

where: d_P, biocatalyst particle diameter (m); D_{SL}, liquid phase diffusivity (m/s); v_S, liquid superficial velocity (m/s); ϕ, volumetric fraction of biocatalyst particles (–); η_L, liquid phase viscosity (Pa s); ρ_L, liquid phase density (kg/m^3).

The liquid phase superficial velocity can be determined using the impeller radial flow rate, Q_{RL}. Thus, for the Rushton turbine with the diameter varying between the limits $0.2 < d/D < 0.4$, the following relationship becomes valid for liquid flow through the cylindrical section [12]:

$$v_s = 0.75 \cdot \frac{N \cdot d^3}{(1-\varphi)\cdot \pi \cdot R \cdot H} \tag{10}$$

In Eq. 10 the following symbols were used: D, bioreactor diameter (m); d, impeller diameter (m); H, basket bed height (m); N, rotation speed (s^{-1}); R, basket bed radius (m).

The variation of liquid superficial velocity is indicated in Fig. 1, assuming that the radial flow rate is constant. For all biocatalyst particle sizes studied, the value of liquid superficial velocity inside the biocatalysts' bed is higher than that corresponding to the outer region of the basket due to the reduced area of flow section. Radial decrease of the superficial velocity through the basket bed is the result of the increased area of the cylindrical flow section.

The highest liquid superficial velocity is reached for the particles of immobilized *A. succinogenes* cells with 3-mm diameter due to the smallest void fraction of the fixed bed (the volumetric fractions of the biocatalysts in the basket bed are as follows: $\phi = 0.84$ for particles with 3-mm diameter, $\phi = 0.71$ for particles with 3.6-mm diameter,

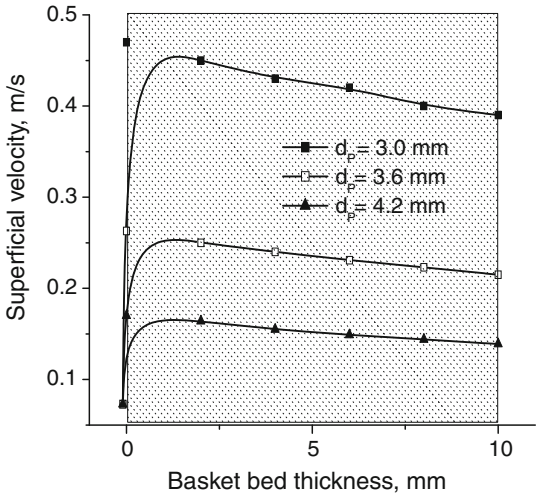

Fig. 1 Variation of liquid-phase superficial velocity inside the basket bed

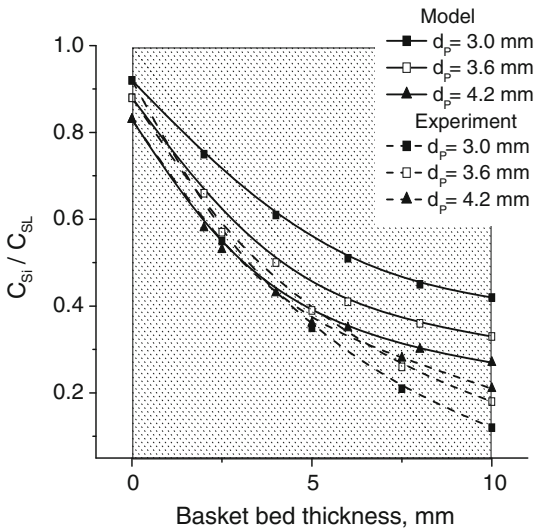

Fig. 3 Variation of ratio C_{Si}/C_{SL} inside the basket bed (C_{Si} substrate concentration at the particle surface, C_{SL} substrate concentration in liquid phase)

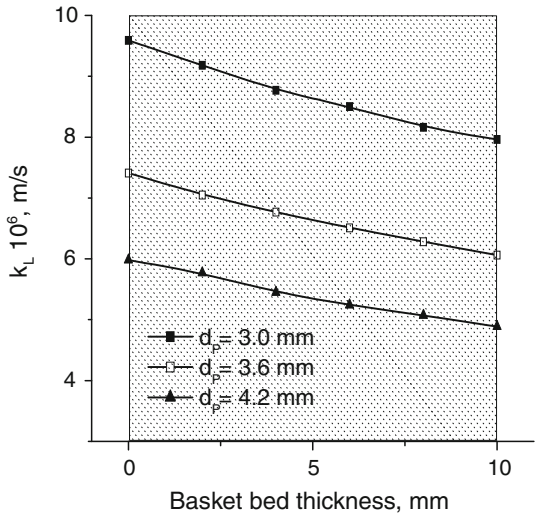

Fig. 2 Variation of glucose mass transfer coefficient inside the basket bed (k_L glucose mass transfer coefficient in the boundary layer at the particle surface)

and $\phi = 0.55$ for the biggest particles). The variation of glucose mass transfer coefficient in the liquid boundary layer at the particle surface is plotted in Fig. 2. Being directly related to the variation of the liquid superficial velocity, the substrate mass transfer coefficient decreases from the inner to the outer surface of the basket bed owing to diminution of turbulence on the same direction. Moreover, compared with the system containing a mobile bed of identical biocatalysts [10], the rate of glucose mass transfer through the liquid boundary layer surrounding the particles for the basket bed is about 8–13 times lower, with this difference increasing with particle size and biocatalyst bed width.

As shown in Fig. 3, the ratio between the substrate concentrations at the particle surface and in the liquid bulk inside the biocatalysts bed decreases radially toward the outer surface of the basket bed. This variation is the consequence of decreasing substrate mass transfer rate through the liquid boundary layer cumulated with its consumption by the immobilized bacterial cells. Figure 3 shows deviations of the experimental data from the model as the result of amplified resistance to substrate diffusion inside the basket bed and of its advanced consumption due to increased residence time inside the basket bed.

These phenomena become more important for the smallest biocatalysts owing to the smallest void fraction of the fixed bed. The growth of *A. succinogenes* in the next cycles of succinic fermentation will lead to the supplementary reduction of the C_{Si}/C_{SL} ratio.

The external mass flow of glucose has been calculated by introducing the values of the above discussed parameters in Eq. 8, its variation on radial direction inside the basket bed being plotted in Fig. 4. Figure 4 emphasizes the contrary variation of mass flow compared with that of the mass transfer coefficient, suggesting that glucose transfer through the boundary layer surrounding the biocatalyst particles is controlled not by its diffusivity but by its concentration gradient between the liquid phase and the particle surface. The faster consumption rate of substrate with the increase of basket-bed thickness leads to

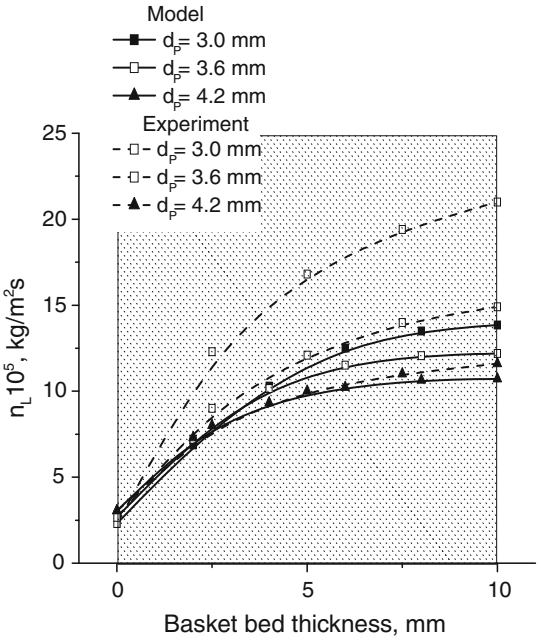

Fig. 4 Variation of external glucose mass flow inside the basket bed (n_L substrate mass flux in the boundary layer at the particle surface)

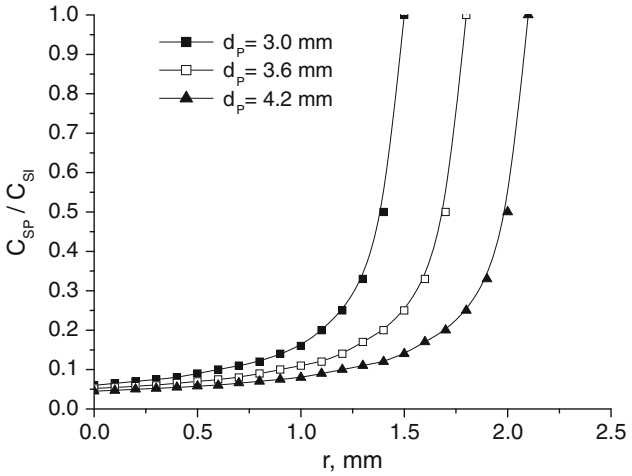

Fig. 5 Variation of ratio C_{SP}/C_{Si} with distance from the particle center (C_{SP} substrate concentration inside the biocatalyst particle, C_{Si} substrate concentration at the particle surface)

amplification of its concentration gradient from the inner to the outer cylindrical surface.

According to those above discussed, due to the pronounced increase of substrate concentration gradient between the liquid phase and the particle surface, deviations between calculated and experimental values of glucose mass transfer are amplified by increasing the cylindrical bed width and by reducing the biocatalysts particle diameter. Thus, the real values of external mass flow are higher than those obtained using Eq. 8. In fact, the model fits with the experimental results only for the largest particles of immobilized bacterial cells.

Circulation velocity of the liquid phase around the particles in the bioreactor with a packed bed of immobilized yeasts cells is significantly slower that that obtained for a mobile bed. Therefore, the substrate mass flow for the basket bed becomes about 1.4–14 times lower than the values corresponding to the mobile bed for the same fermentation conditions [10]. Reduced external mass flow becomes more important with decreased biocatalyst particle size and increased cylindrical bed thickness.

The internal mass flow can be obtained by combining the Fick law:

$$n_P = -D_{Se} \cdot \frac{dC_{SP}}{dr} \tag{11}$$

with Eq. 4, resulting in the following expression, which is adequate for this system of succinic fermentation under substrate and product inhibition:

$$n_p = D_{Se} \cdot \frac{Bi \cdot (C_{SL} - C_{Si}) \cdot \cosh(3\varphi \cdot R_p)}{R_P^3} \cdot \left[3\varphi - R_p^2 \cdot \tanh(3\varphi \cdot R_p)\right] \cdot \left[\frac{3\phi \cdot \cosh\left(\frac{3\varphi \cdot r}{R_p}\right)}{R_p \cdot r} - \frac{\sinh\left(\frac{3\varphi \cdot r}{R_p}\right)}{r^2}\right] \tag{12}$$

Because the glucose concentration inside the biocatalyst particle depends strongly on its concentration at the particle surface, variation of the ratio between these two concentrations with the particle radius for the three considered diameters could describe more accurately the influence of internal diffusion. In this context, for all considered sizes of biocatalyst particles, from Fig. 5 it can be seen that the ratio C_{SP}/C_{Si} is significantly reduced toward the particle center, an effect that is more important for the larger particles. Thus, the concentration ratio decreases from 1 at the particle surface to different values in the particle center, depending on the diameter of biocatalysts (0.06 for the biocatalysts with 3-mm diameter, 0.052 for biocatalysts with 3.6-mm diameter, and 0.045 for the largest biocatalysts). This order is not affected by the position inside the basket bed.

Therefore, depending on substrate concentration at the particle surface, position inside the biocatalysts bed, and, respectively, biochemical process rate, the glucose concentration in the particle center could reach a very low value compared with that in the liquid bulk, this becoming an important threat for the normal succinic fermentation process (Figs. 6, 7). Although for the first half of the basket-bed thickness these influences are more important for the largest biocatalyst particles, their relative magnitude is changed by increasing the basket-bed thickness. Therefore,

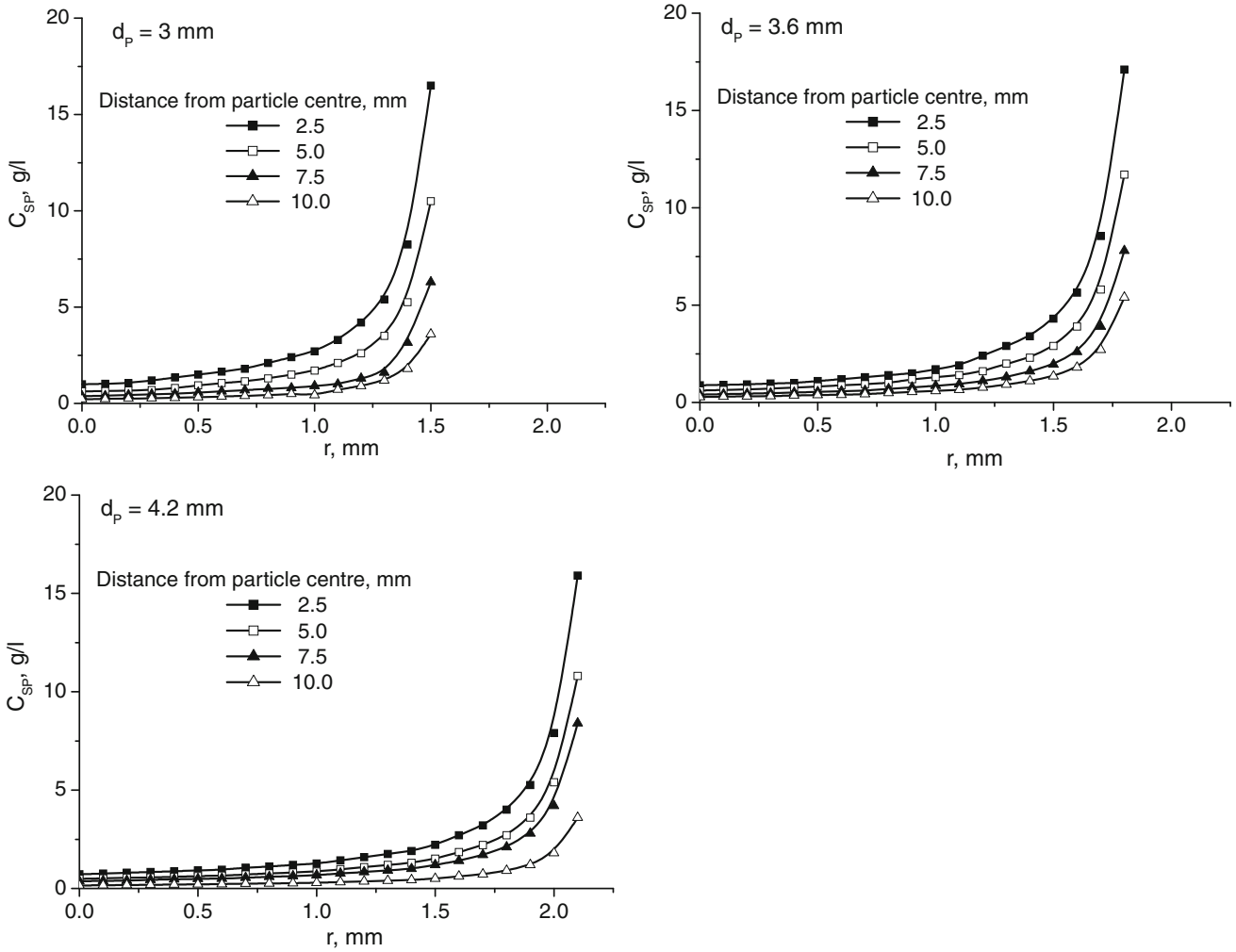

Fig. 6 Variation of glucose concentration inside the biocatalyst with distance from the particle center (C_{SP} substrate concentration inside the biocatalyst particle)

due to the superior resistance to the glucose diffusion inside the cylindrical bed and consumption rate, at the outer surface of the basket bed, the lowest substrate concentration in the particle center is reached for the smallest biocatalysts (Fig. 7).

By means of Eq. 12, the values of the internal mass flow of glucose were estimated.

By comparing values of the internal mass flow of glucose with those of its external mass flow, it can be observed that the first ones are about 10^5 times lower. Moreover, Fig. 8 emphasizes the direct correlation between the internal mass flow and substrate concentration, both being significantly reduced at the particle center. This reduction becomes more pronounced with the increase of the size of immobilized *A. succinogenes* cell particles.

Variation of the substrate mass flow with the biocatalyst particle radius suggests that it is possible to reach very low or negligible values of mass flow near the particle center.

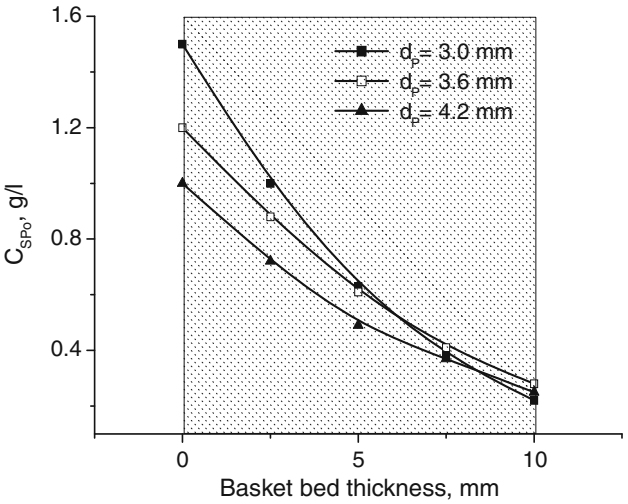

Fig. 7 Variation of glucose concentration in the particle center with the particle position inside the basket bed (C_{SPo} substrate concentration in the particle center

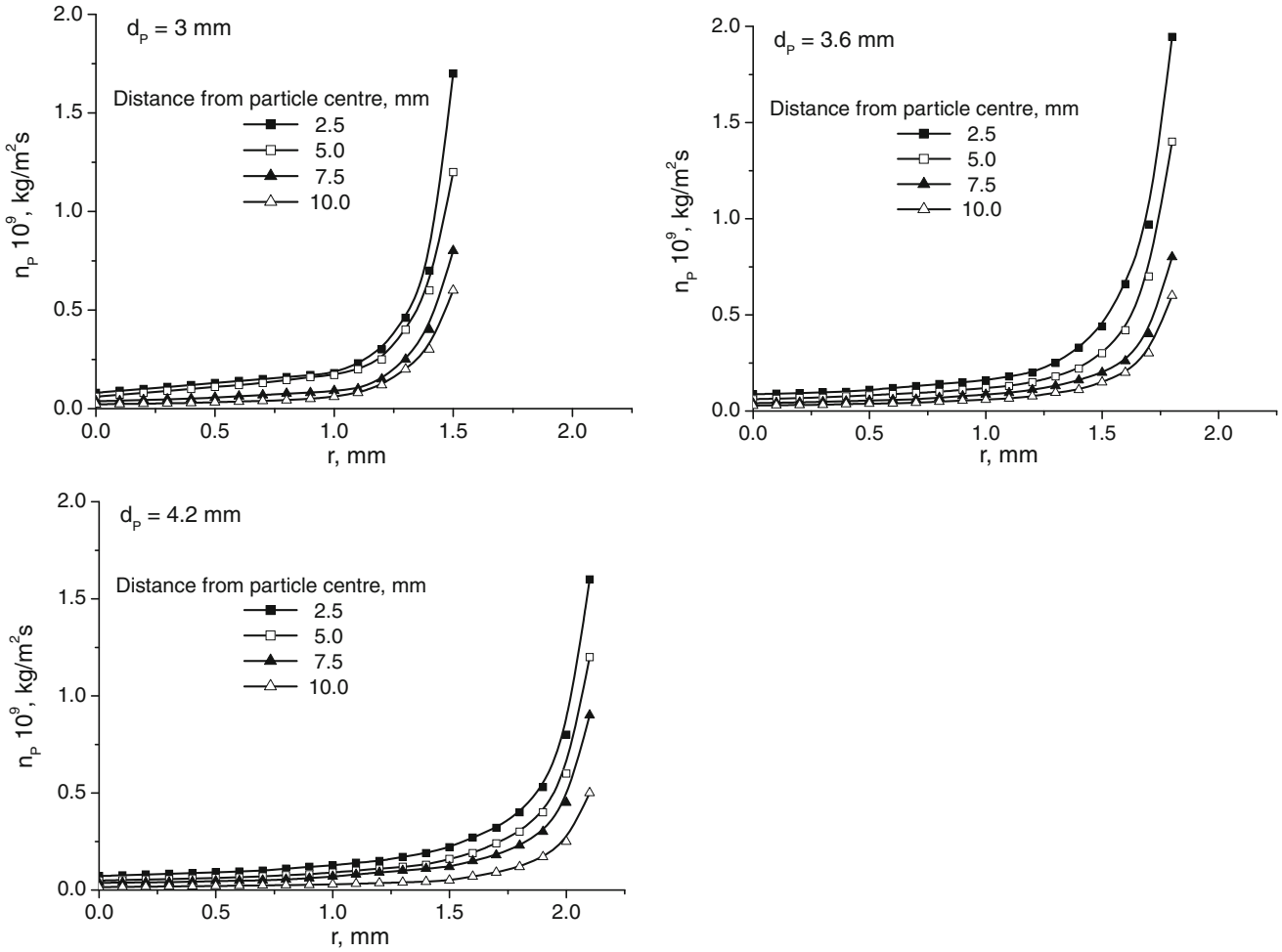

Fig. 8 Variation of internal glucose mass flow with distance from the particle center (n_L substrate mass flux inside the biocatalyst particle)

This region could be considered as a biologically inactive region. Taking into consideration the order of magnitude of effective diffusivity, it can be assumed that for values of internal mass flow $<10^{-10}$ kg/m^{2}s, the mass transfer of glucose becomes insignificant [10]. Therefore, according to Fig. 9, the extent of the biologically inactive region varies from 0.24% to 44% from the overall volume of each biocatalyst particle, the highest values being recorded for the largest particles at the outer surface of basket bed.

For any distance from the particle surface to its center and for basket-bed thickness >5 mm, the highest values of internal mass flow of glucose are reached for the intermediary biocatalyst particles as the consequence of the equilibrium between diffusion rates in the bed and inside the biocatalysts. This result cumulated with those obtained for the mobile bed [10] indicates the particles diameter of 3.6 mm as the optimum size of biocatalysts. The extent of the inactive region is important on the radial direction inside the basket bed. Thus, near the outer surface of the cylindrical bed, the volume of the inactive region becomes about eight times greater than in the case of mobile-bed

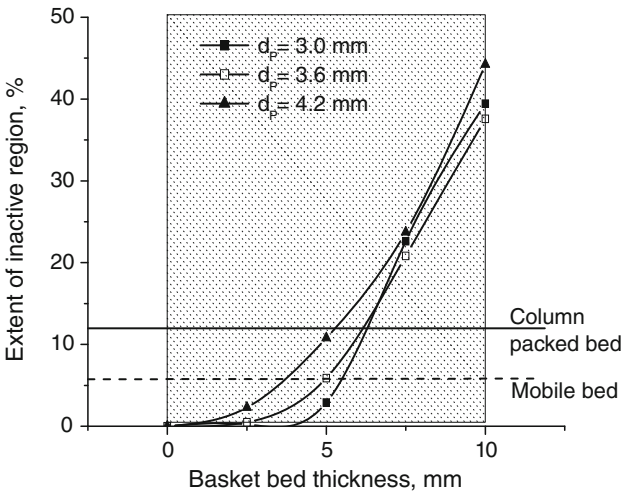

Fig. 9 Variation of extent of biologically inactive region inside the basket bed

immobilized cells. In particular, only for the first half of the basket-bed thickness is the extent of this region similar to that corresponding to the mobile bed. However, compared

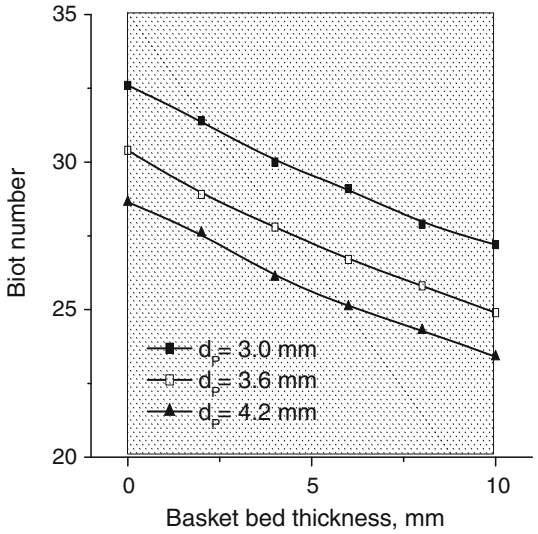

Fig. 10 Variation of Biot number inside the basket bed

with the column bioreactor with a packed bed of biocatalysts [32], the extent of this region is lower in the first 60% of the basket-bed width from the inner surface. As presented above, Eqs. 4 and 5 include two parameters that quantify the influence of internal diffusion either on the transfer process or on the biochemical reaction rate: the Biot number, *Bi*, and the Thiele modulus, φ. In this context, the relative importance of the glucose diffusion processes in the liquid boundary layer surrounding the particle and inside the particle, described by the *Bi* number, is controlled by the size and concentration of biocatalyst particles. Therefore, from Fig. 10 it can be observed that this number is diminished radially toward the outer surface of the basket bed, the highest values being reached for the smallest biocatalyst particles. This variation is in concordance with the dependence between glucose mass transfer through the liquid phase and basket-bed thickness or particle size.

By comparing the average values of Biot number in the cylindrical bed for each experimented biocatalyst particle size ($Bi = 29.7$ for biocatalysts with 3-mm diameter, $Bi = 27.9$ for biocatalysts with 3.6-mm diameter, and $Bi = 25.8$ for biocatalysts with 4.2-mm diameter) with those recorded for the mobile bed of immobilized *A. succinogenes* cells with the same diameters ($Bi = 275$ for biocatalysts with 3-mm diameter, $Bi = 301.2$ for biocatalysts with 3.6-mm diameter, and $Bi = 325.1$ for biocatalysts with 4.2-mm diameter [10]) reduction of Biot number for the basket bed of about 9–13 times can be observed, with the most important effect corresponding to the largest particles. This difference is the consequence of the significant reduction of substrate mass transfer coefficient in the liquid boundary layer at the particle surface, which is due to diminution of turbulence and to the appearance of supplementary resistance induced by substrate diffusion inside the fixed bed. Values of the Thiele modulus are specific for a given size of immobilized bacterial cell particles do not vary within the basket bed and are similar to those calculated for the mobile bed ($\varphi = 0.026$ for biocatalysts with 3-mm diameter, $\varphi = 0.032$ for biocatalysts with 3.6-mm diameter, and $\varphi = 0.037$ for biocatalysts with 4.2-mm diameter). For describing more accurately the effect of the internal diffusion on the rate of glucose consumption during succinic fermentation, the reduction factor λ is used [9]. This factor is defined as the ratio between the rates of biochemical reaction in heterogeneous and homogeneous systems. Considering the steady-state conditions, it can be assumed that the rate of the internal biochemical reaction equals the internal mass flow of glucose. Therefore, for the investigated succinic fermentation system, the relationship for calculating the factor λ becomes (Eq. 13):

$$\lambda = \frac{4\pi \cdot R_P^2 \cdot D_{Se} \cdot \frac{dC_{SP}}{dr}/r = R_P}{\frac{4}{3}\pi \cdot R_P^3 \cdot V \cdot C_C \cdot \left(\frac{K_{iS}}{K_{iS}+C_S}\right) \cdot \left(\frac{K_{iP}}{K_{iP}+Y_{P/S}\cdot C_S}\right)} \tag{13}$$

By combining Eqs. 4 and 13, the following relationship for calculating the factor λ is obtained:

$$\lambda = \frac{3 \cdot k_L \cdot (C_L - C_{Si}) \cdot \cosh(3\varphi \cdot R_P) \cdot \left(\frac{3\varphi}{R_P} - R_P \cdot \tanh(3\varphi \cdot R_P)\right) \cdot \cosh(3\varphi) \cdot [3\varphi - \tanh(3\varphi)]}{R_p^4 \cdot V \cdot C_c \cdot \left(\frac{K_{iS}}{K_{iS}+C_S}\right) \cdot \left(\frac{K_{iP}}{K_{iP}+Y_{P/S}\cdot C_S}\right)} \tag{14}$$

The variation of factor λ with the particle radius is graphically presented in Fig. 11. Analysis of the dependences plotted for each diameter of biocatalyst particles indicates that in all cases, this factor varies slowly near the particle surface or center. In the region in the vicinity of the biocatalyst surface, the higher concentration of substrate, wich is rather equal to that at the particle surface, leads to the values of λ close to 1. This layer thickness

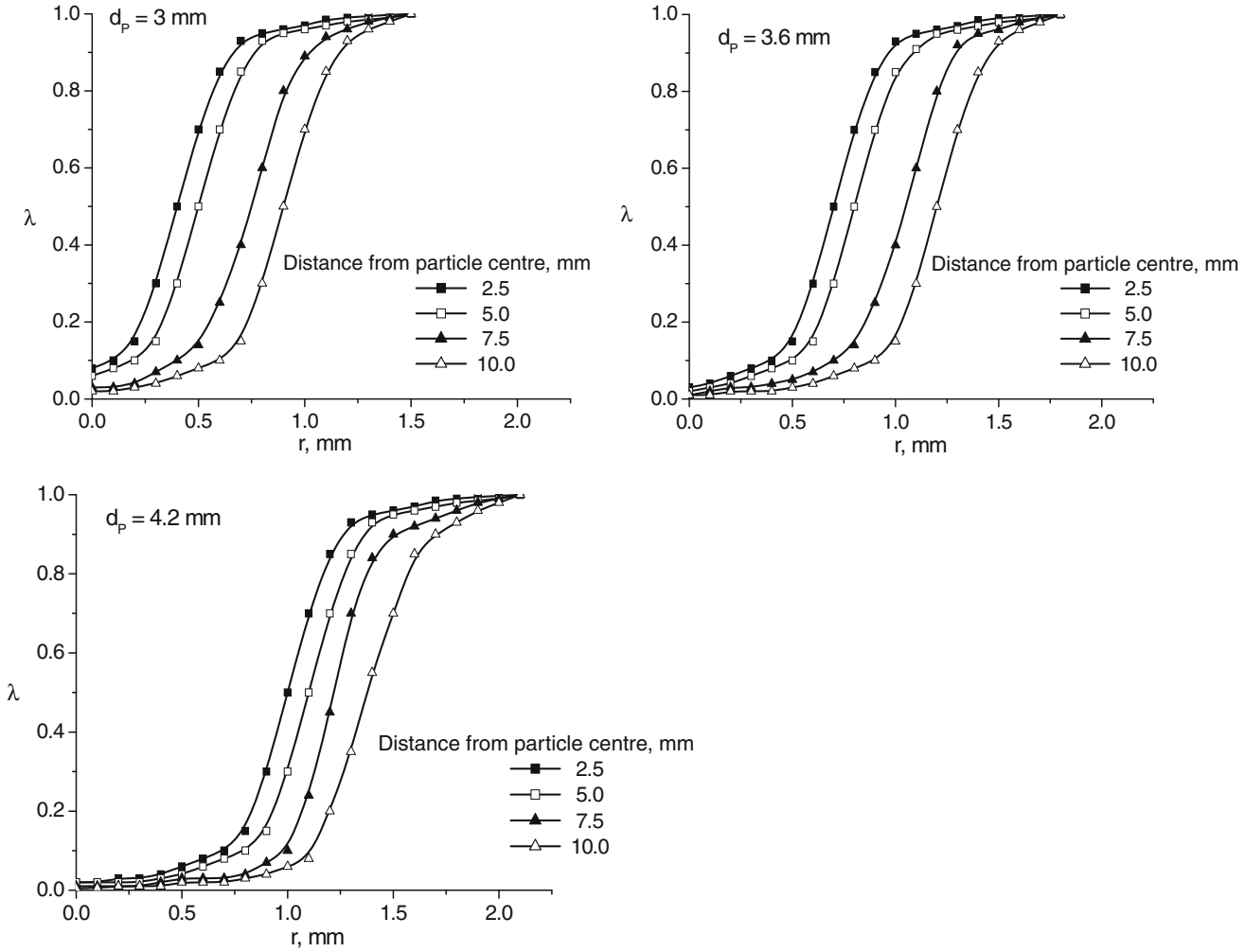

Fig. 11 Variation of reduction factor λ with distance from the particle center

corresponding to the slow diminution of λ (from 1 to 0.9) does not depend on particles size but only on position inside the basket bed.

The slow variation of factor λ in the central region is the result of the constant low level of glucose concentration near the particle center (Fig. 6). In direct relation with the variation of substrate concentration inside the particle, the thickness of the intermediary region corresponding to the significant reduction of λ from its superficial value to the central one is extended with the increase of particle size and basket-bed width. Thus, it can be concluded that by using the fixed bed of basket conformation containing immobilized *A. succinogenes* cells, the rate of the biochemical glucose conversion is considerably reduced for $1/\lambda$ times compared with that reached for free bacterial cells ($1/\lambda$ is about 200 times by with the succinic fermentation system containing free cells [15]). The magnitude of this effect must be correlated with the size and position inside the bed of the

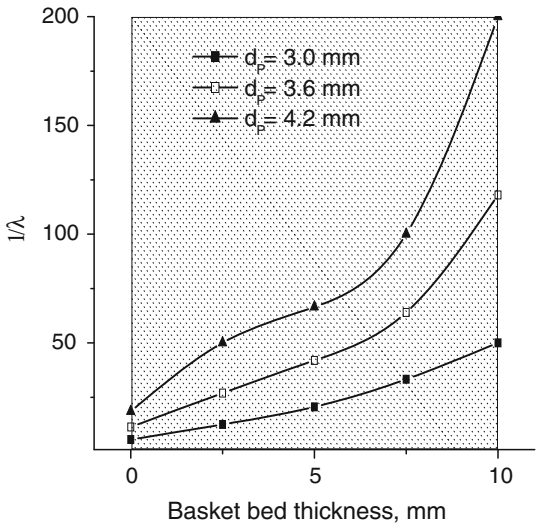

Fig. 12 Variation of parameter $1/\lambda$ inside the basket bed

biocatalyst particles. Thus, for those reasons, the parameter $1/\lambda$ is increased from the inner to the outer surface of cylindrical bed of about 8.9 times for the smallest particles, 10.3 times for the intermediary ones, and 10.8 times for the largest particles (Fig. 12). Reduction-effect amplitude on glucose consumption rate increased only four times for succinic fermentation carried out in a bioreactor with mobile-bed immobilized *A. succinogenes* cells [10].

Conclusions

We analyzed succinic fermentation using a bioreactor with a stationary basket bed of immobilized *A. succinogenes* cells on alginate under substrate and product inhibition conditions. The studies focused on the external and internal mass transfer of substrate and, implicitly, the influence of internal diffusion on transfer and biochemical process rates. Results are discussed in direct relation to the biocatalyst particle size and position inside the basket bed. Specific mathematical models were developed, taking into account the substrate mass balance for a single particle under inhibitory effects and was used to calculate glucose concentrations at the surface and inside the biocatalyst. Using these concentration values, we estimated glucose mass flows through the liquid boundary film surrounding and inside the particles. We found that the values of external mass flows are about 1.4–14 times lower than those obtained for the mobile bed of the same biocatalysts, the difference becoming more important with the decrease of biocatalyst particle size and increase in cylindrical-bed thickness. The variation of internal mass flow of glucose with particle radius indicates that it is possible to reach very low values of mass flow close to the particle center, which are values that could be negligible. The region inside the particle corresponding to the insignificant mass transfer of glucose could be considered a biologically inactive region, its magnitude varying from 0.24% to 44% from the overall volume of each biocatalyst particle size studied. The highest extension of this inactive region corresponds to the largest particles at the outer surface of the basket bed. Furthermore, the influence of the substrate's internal diffusion on the biochemical process rate was analyzed using the Biot number, the Thiele modulus, and reduction factor λ. Biocatalyst particle size and position inside the basket bed exhibit significant influences on the above-mentioned parameters. Therefore, increase in particle diameter led a decrease in Biot number and Thiele modulus and, implicitly, to a decrease in factor λ in the particle center. From the inner to the outer surface of the basket bed, the glucose consumption rate decreased about 8.9–10.8 times, depending on biocatalyst particle diameters.

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